Effects of Oral Lentinan on T-Cell Subsets in Peripheral Venous Blood

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ABSTRACT

The effect of oral lentinan, a biological response modifier, on the control of systemic immune function was studied in six-week-old male Wistar-Imamichi specific-pathogen free rats. In the lentinan-treated group, 1 mg of lentinan dissolved in 1 ml of physiological saline was administered forcibly into the stomach twice weekly for four or eight weeks. Physiological saline alone was administered in a similar fashion to the control group. Leukocyte and lymphocyte counts were made and lymphocyte subsets measured using monoclonal antibodies W3/13, W3/25, and OX8, and a laser flow cytometry system. The T-cell level, the helper/inducer T-cell level, and the suppressor/cytotoxic T-cell level were measured. The peripheral leukocyte and lymphocyte counts did not change significantly in either group during treatment. After four weeks of treatment, however, the lentinan group had a significantly higher T-cell level, helper-cell level, and helper-suppressor ratio, and a significantly lower suppressor-cell level than did the control group. No significant between-group differences in the lymphocyte subsets or the helper-suppressor ratio were noted after eight weeks of treatment. Oral administration of lentinan appears to modulate the systemic immune function through stimulation of T cells, especially helper cells. Continued administration produced less effect, possibly due to a tolerance to the effect of lentinan.

INTRODUCTION

Lentinan (eritadenine) is a glucan with a molecular weight of 950,000 to 1,050,000 daltons that was extracted from *Lentinus edodes* by Chihara and coworkers[1] in 1969. This compound inhibits the growth of sarcoma-180 when transplanted into the subcutaneous tissue of mice, and its use as a biological response modifier in nonspecific immunotherapy for cancer has been studied.[2]

Lentinan is distinguished from other response modifiers currently in use by the absence of a direct cytotoxic action, despite its antitumor effects on various congenic tumors.[2,3] This may be explained by the characteristics of a neutral polysaccharide; lentinan is a biological response modifier with a true host-mediated antitumor effect. In addition to the absence of tumor cytotoxicity, lentinan exerts no unfavorable effects on normal cells; thus, in contrast to other anticancer agents, side effects of lentinan treatment are absent.

A positive effect of lentinan on the survival of patients with advanced or recurrent cancer of the digestive organs has been demonstrated in a randomized, prospective, controlled study.[4] Lentinan was administered intravenously over as long a period of time as possible, and may have had a life-prolonging effect on the patients.[5] However, when evaluating the quality of life of cancer patients, the discomfort of repetitive intravenous administration, and the mental and physical stress associated with frequent hospitalization must not be underestimated.

The simplest and most comfortable method of drug administration is the oral route. Although there have been few studies of the oral administration of lentinan,[6] the oral administration of other biological response modifiers has been studied. Tsuchiya and coworkers[7] studied the effects of an oral administration of OK-432, a hemolytic streptococcal preparation, and bacille Calmette-Guérin vaccine. An immunoactivating effect of these drugs was demonstrated throughout the host, mediated by the gut-associated lymphoid tissue. OK-432 administration was also reported[7] to cause a decrease in the size of experimental cecal cancers in mice with prolonged survival time. In our studies[8] of oral OK-432, changes of the lymphocyte subsets in the thoracic duct lymph and in peripheral blood were demonstrated.

In the present study, lentinan was administered orally and changes in the lymphocyte subsets in the peripheral venous blood and its effects on systemic immune function were evaluated.

MATERIALS AND METHODS

Male Wistar-Imamichi specific-pathogen free (SPF) rats aged six weeks were used. In the lentinan group, 1 mg of lentinan, dissolved in 1 ml of physiological saline, was forcibly administered into the stomach through a stainless steel cannula twice a week. In the control group, physiological saline was administered in a similar fashion. The animals were divided into four groups of ten rats each: one group received lentinan for four weeks (a total of 8 mg in eight administrations), one group received lentinan for eight weeks (a total of 16 mg in 16 administrations), and two control groups received

physiological saline for four and eight weeks, respectively.

Animals from each group were sacrificed using an intraperitoneal injection of 6 mg pentobarbital sodium per 100 gm, two days after the final day of administration. This was followed by laparotomy and venous blood sampling by direct puncture of the vena cava, using 5% edetic acid solution as an anticoagulant. A general blood count and differential leukocyte count were performed, followed by lymphocyte subset determinations on the remainder of the blood sample.

Using a method previously reported,[9] lymphocyte subsets were determined using monoclonal antibodies W3/13, W3/25, and 0X8 (Sera-Lab, Crawley Down, Sussex, England). As a negative control, mouse IgG in a 20-fold dilution was used instead of the monoclonal antibody. Cells positive for each monoclonal antibody were detected using an indirect method. Goat antimouse IgG antibody labeled with fluorescein isothiocyanate (FITC Conjugated Goat Anti-Mouse IgG, Ortho Diagnostic Systems, Inc, Raritan, New Jersey) was used as the secondary antibody.

Measurements were made with a laser flow cytometry system (Orthospectrum III, Ortho Diagnostic Systems, Inc), with an argon ion laser (wavelength, 488 nm) as the light source, and anterior scatter, 90-degree scatter, and green fluorescence of 515.5 nm to 620 nm wavelength for optical information on the cells. The precision of measurement with this laser flow cytometry system was 1% for the number of particles and under 1% for the mean signal intensity for each measure. The T-cell level, the helper/inducer T-cell level, and the suppressor/cytotoxic T-cell ratio were measured.

The data were analyzed for significance using Student's *t* test or paired *t* test.

RESULTS

There were no significant changes in the peripheral leukocyte or lymphocyte count after four and eight weeks of treatment in either the lentinan or control groups, and no significant between-group differences were noted (table).

The T-cell level was significantly higher after four and eight weeks of treatment than before treatment in both groups (Figure 1). At four weeks, the T-cell level was significantly higher in the lentinan group than the controls. No significant between-group differences were noted at eight weeks.

The helper-cell level was significantly higher after four and eight weeks of treatment than before treatment in the lentinan group, and significantly lower at eight weeks than at four weeks (Figure 2). No significant changes were noted at four weeks in the control group; however, the helper-cell level at eight weeks was significantly higher than before treatment and at four weeks. At four weeks, the level in the lentinan group was significantly higher than in the control group. No significant between-group differences were noted at eight weeks.

The suppressor-cell level was unchanged in the lentinan group during treatment (Figure 3). In the control group the level tended to rise at four weeks and was significantly higher at four weeks than at eight weeks. At four weeks the suppressor-cell level was significantly higher in the control group than in the lentinan group. No significant between-group differences were noted at eight weeks.

The helper-suppressor ratio was significantly higher after four and eight weeks than before treatment in the lentinan group (Figure 4). In the control

Table. Mean (±SD) changes in leukocyte and lymphocyte counts in the peripheral venous blood of rats after four weeks or eight weeks of treatment with lentinan, versus controls.

	Before Administration (n = 10)	After 4 Weeks of Administration		After 8 Weeks of Administration	
		Lentinan (n = 10)	Control (n = 10)	Lentinan (n = 10)	Control (n = 10)
Leukocyte count ($\times 10^3/\mu L$)	7.0 ± 0.9	8.0 ± 1.8	8.8 ± 2.4	8.8 ± 2.4	6.9 ± 1.4
Lymphocyte count ($\times 10^3/\mu L$)	6.0 ± 0.8	6.3 ± 0.9	6.5 ± 1.8	7.2 ± 1.9	6.2 ± 1.4

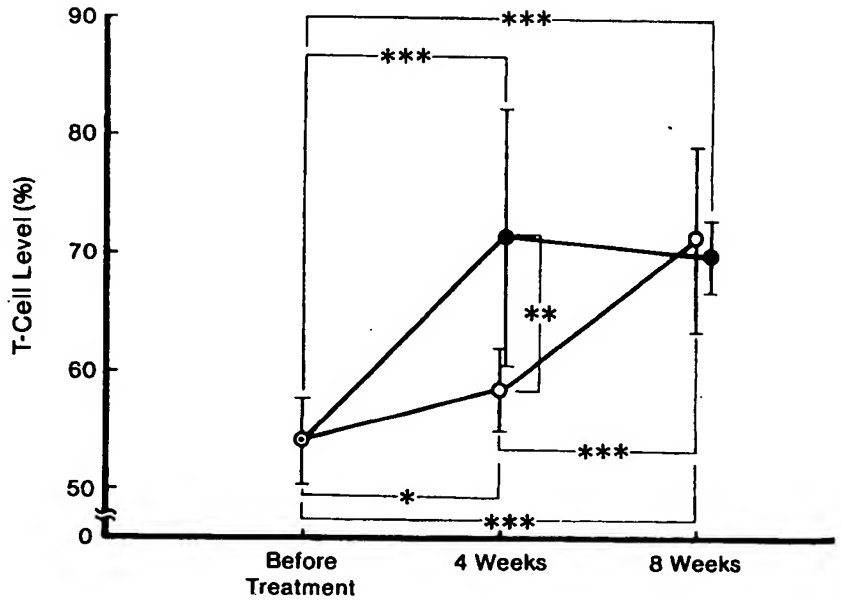

Figure 1. Mean (±SD) T-cell levels in lentinan-treated rats (filled circles) and control rats (open circles) before and after four and eight weeks of oral administration. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

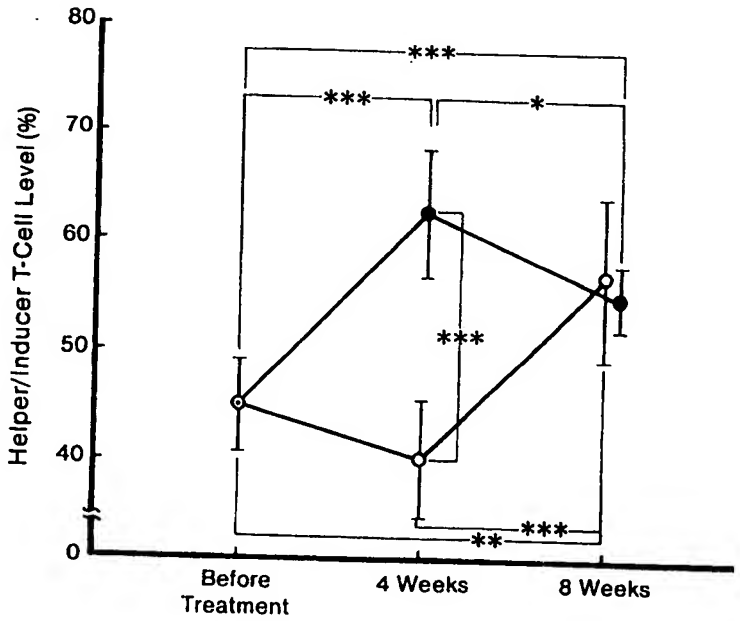

Figure 2. Mean (±SD) helper/inducer T-cell levels in lentinan-treated rats (filled circles) and control rats (open circles) before and after four and eight weeks of oral administration. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

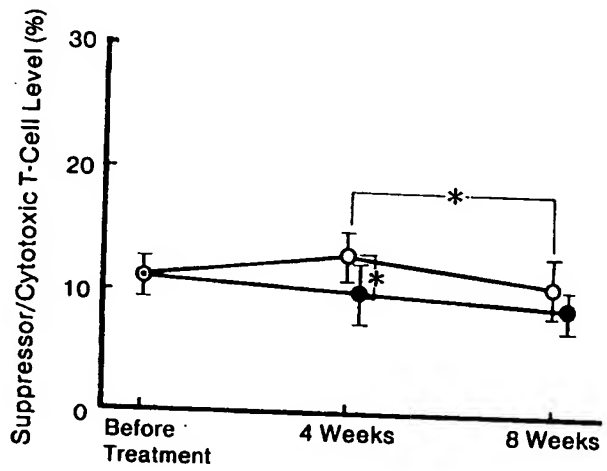

Figure 3. Mean (±SD) suppressor/cytotoxic T-cell levels in lentinan-treated rats (filled circles) and control rats (open circles) before and after four and eight weeks of oral administration. $^{*}P < 0.05$.

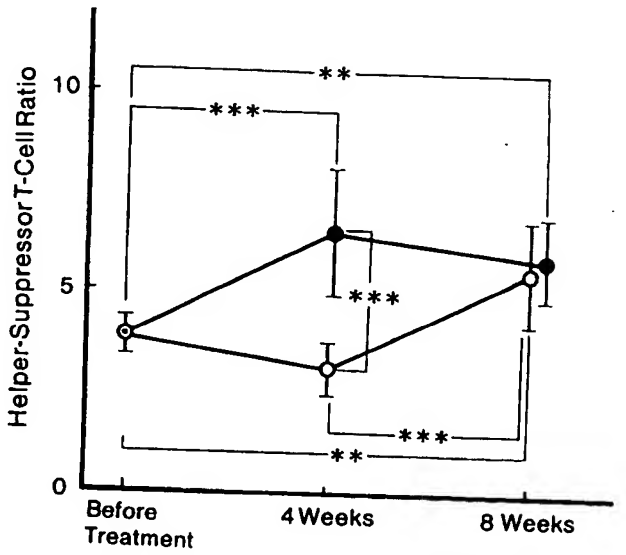

Figure 4. Mean (±SD) helper-suppressor T-cell ratios in lentinan-treated rats (filled circles) and control rats (open circles) before and after four and eight weeks of oral administration. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

group, the ratio was significantly higher after eight weeks of treatment than before treatment and higher at eight weeks than at four weeks. At four weeks, the helper-suppressor ratio was significantly higher in the lentinan group than in the control group. No significant between-group differences were noted at eight weeks.

DISCUSSION AND CONCLUSIONS

Lentinan is a biological response modifier that modulates the immune system through activation of thymic lymphocytes, especially helper cells.[1,2] It was reported[3] in healthy animals to normalize the immune function, suppressed by some disease processes, without increasing the immune function to supernormal levels.

According to Takatsu and associates,[10] lentinan, in mice, prevents the antibody memory cell dysfunction produced in response to tumor cell transplantation. This suggests a normalizing action of lentinan on the ability to produce humoral antibody in the immunocompromised cancer-bearing animal. However, Maeda and Chihara,[3] using sheep erythrocytes as the antibody, failed to demonstrate an effect of lentinan on humoral antibody production in noncancer-bearing mice.

Haba and coworkers[11] reported the inhibition of T-cell activity in cancer-bearing mice and an increased activity after lentinan administration. Shio and associates[12] reported that lentinan protected cancer-bearing mice from decreased cell-mediated immunity without elevating cell-mediated immunity to supernormal levels in noncancer-bearing animals.

In the present study of the effects of oral lentinan administration, lymphocytes in peripheral venous blood were used as an index of the immune function of the whole body. SPF rats were studied, thus eliminating the confounding variable of the influence of infection.

No definite trend was noted in the peripheral leukocyte and lymphocyte counts after the oral administration of lentinan for four weeks. However, in the lymphocyte subsets, the lentinan group demonstrated a significantly higher T-cell level, helper-cell level, and helper-suppressor ratio, and a significantly lower suppressor-cell level than did the control group. These results indicate that lentinan did not change the total lymphocyte count; rather, it increased the proportion of helper cells relative to that of suppressor cells, thereby activating the immune function of the T-cell system.

Lymphocyte function was not measured; however, these results are compatible with those reported by Dennert and Tucker[13] and Dresser and Phillips[14] regarding stimulation of helper-cell activity after intraperitoneal administration of lentinan in normal animals. The oral administration of lentinan probably exerted a modifying effect on the immune system of the body as a whole via the gut-associated lymphoid tissue. The effect, however, disappeared after eight weeks of administration.

Lentinan did not cause any serious injury to the organs in a general pharmacological test.[15] In the present study, the rats receiving lentinan remained as healthy as those in the control group, with no differences in body weight. Lentinan did not appear to cause damage to the rats' immune function.

The oral administration of an antigen generally decreases reactivity to systemic administration, a phenomenon known as oral tolerance.[16,17] While the mechanism of oral tolerance has not been completely elucidated, participation of suppressor cells,[18] the anti-idiotypic network,[19] and immune complex formation[20] are suspected. Suzuki et al[21] demonstrated suppressor-cell inhibition by contrasuppressor effector cells in the prevention of oral tolerance. Oral tolerance probably represents a self-preserving function in animals with normal immune function to maintain homeostasis by inhibiting excessive defense mechanism responses to exogenous antigen.[22]

The absence of an immunomodulatory effect after eight weeks of oral lentinan may be due to a tolerance to lentinan in rats with normal immune function. Comparative experiments using rats with immune dysfunction are thus necessary.

The most favorable clinical application of a biological response modifier is as adjuvant treatment in long-term immunotherapy after surgical resection of a tumor.[23] Since the immune system remains normal in these patients, the appearance of tolerance, which can occur with normal immune function, presents a major problem. Many of the previous studies of the oral administration of biological response modifiers have been conducted over relatively short periods.[24,25] The long-term effects of biological response modifiers on immune function have seldom been studied, and thus data on the phenomenon of tolerance are insufficient.

In the present study, changes in lymphocyte subsets were compared in lentinan-treated and control groups after eight weeks of administration. Since changes in lymphocyte subsets by age in normal rats have never been established, it was not possible to distinguish environmental or age-related influences on the rise in the T-cell and helper-cell levels or on the fall in the suppressor-cell level in the control group. Information on physiological changes in indices of immune function in rats will be needed for future studies in this area.

Because the oral administration o lentinan modifies the immune functio of the entire body, this route may b useful as an immunotherapy. Becaus tolerance appears after continued admin istration, modification of the administra tion protocol or the type of preparatio should be considered.

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Dantrolene Sodium in Traumatic Muscle Contracture: Double-Blind Clinical and Pharmacological Trial

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ABSTRACT

Thirty athletes with muscular contractures were enrolled in a double-blind study of dantrolene sodium and placebo to evaluate the decontracture activity and tolerance of the drug after eight days of treatment. The efficacy of the drug was assessed by studying pain at rest, during movement, and during pressure, as well as muscular tension and functional recovery.

Twenty-eight patients completed the study. At the end of treatment, a decrease in pain was observed at rest (71.4% of patients treated with dantrolene and 21.4% of placebo-treated patients), during movement (78.6% and 35.7%, respectively), and during compression. The most noticeable effects were seen in the reduction of muscular tension (100% in the patients treated with dantrolene sodium and 35.7% in the placebo-treated patients) and in functional recovery (100% and 28%, respectively).

In addition to the clinical study, an evaluation of the effects of dantrolene and placebo on overall performance and on the action of the respiratory system was conducted with six healthy subjects by means of basal respiratory measurement and ergospirometry before and after single-dose treatment.

This study showed that dantrolene sodium is useful in the treatment of traumatic contracture, and that it does not alter an individual's overall performance. Dantrolene sodium represents a valid treatment to accompany analgesic, anti-inflammatory, and rehabilitation therapy of posttraumatic lesions in athletes.

INTRODUCTION

Athletic activity may result in injury to the trunk and limbs, including muscle pulls, dislocations, and fractures.[1,2] The most common injuries of the lower limbs involve the muscle, the muscle-tendon joint, the tendon, and the tendon-periosteum joint.[1] Injuries to these areas may be direct (contusion or muscle pulls) or indirect (inflammation of the tendon sheath, bursa, or ligament). Direct injuries are related mainly to violent sports